

Appl. No. 09/926,808
Amendment dated: September 7, 2004
Reply to FR of May 5, 2004

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims:

1(currently amended). Reverse transcriptase (RT) assay kit comprising one or several package(s) containing solid phase bound polyriboadenylic acid (prA) and/or polydeoxyadenylic acid (pdA) template(s) ~~obtainable~~ produced by contacting coupling prA and/or pdA to a polystyrene-based solid phase with a coupling solution comprising consisting essentially of 1-methylimidazole, and prA and/or pdA at a pH \approx from about 5-7, followed by incubation, washing with a wash buffer, drying and packaging, RT-type adapted separately packaged assay components selected from the group consisting o a mixture of or separately a buffer, pH \approx 7-8, divalent metal ion, chelator, polyamine, RNase inhibitor, reducing agent, salt, stabilizing agent, and detergent, and a mixture of or separately lyophilized deoxynucleotide triphosphate, primer, protective agent and a concentrated washing buffer, and optionally lyophilized reference enzyme(s), and optionally components of a detection system comprising lyophilized alkaline phosphatase conjugated anti-BrdU monoclonal antibody, alkaline phosphatase substrate buffer and alkaline phosphatase substrate, and written instructions for use of the assay kit.

2(currently amended). RT assay kit according to claim 1, wherein the solid phase is a microtiter plate and an aliquot of the coupling solution, which ~~comprises~~ consists essentially of 100 mM 1-methylimidazole, pH \approx 5-7, and 0.5 - 2 mg/ml prA and/or pdA, is added to each well, followed by the incubation at a temperature of 10 - 60°C for 0.5 - 10 h, and washing each well for the removal of the 1-methylimidazole with the wash buffer, which comprises Bis-Tris propane, pH \approx 5-7, and drying and packaging the plates.

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3(currently amended). RT assay kit according to claim 2, wherein 100 μ l of the coupling solution, which ~~comprises~~ consists essentially of 100 mM 1-methylimidazole, pH \approx 6.25, and 1 mg/ml prA and/or pdA, is added to each well, followed by the incubation at room temperature for \approx 2 h, washing of each well with 2x300 μ l of the wash buffer, which comprises 10 mM Bis-Tris propane, pH \approx 6.25, drying the plates at 37°C for \approx 25 minutes and putting the plates in foil bags and vacuum sealing the bags.

4(previously presented). RT assay kit according to claim 1, wherein the assay components are one or a mixture of the buffers Tris and Hepes, pH \approx 7-8, one or a mixture of the divalent metal ions Mg²⁺ and Mn²⁺, one or a mixture of the chelators ethylenediaminetetraacetic acid (EDTA) and ethylene glycol-bis(β -aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA) and, one or a mixture of the polyamines spermine and spermidine, one or a mixture of the RNase inhibitors heparin sulfate and dextran sulfate, one or a mixture of the reducing agents dithiothreitol (DTT), dithioerythritol (DTE), and glutathione, one or a mixture of the salts NaCl and KCl, one or a mixture of the stabilizing agents newborn calf serum (NCS) and bovine serum albumin (BSA), one or a mixture of the detergents Tween 20 and Triton X-100, the deoxynucleotide triphosphate BrdUTP, the primer oligo dT, and one or a mixture of the protective agent agents ATP, GTP and CTP.

Claims 5-8(canceled).

9(previously presented). Method of qualitative and quantitative analysis of RT activity in a biological sample comprising the steps of using and following the written instructions for the RT assay kit according to claim 1 for the determination of the RT activity in the biological sample.

10(currently amended). Method according to claim 9 wherein the biological sample is selected from the group consisting of biological fluids and cell extracts.

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11(currently amended). Method according to claim 10 wherein the biological fluid is selected from the group consisting of plasma, serum, spinal fluid, synovial fluid and pleural fluid.

12(previously presented). Method according to claim 9 followed by evaluation of the status of a RT activity related disorder or disease based on the result of the analysis of the RT activity.

13(currently amended). A polystyrene-based solid phase support for a Reverse Transcriptase (RT) assay kit, wherein said polystyrene-based solid phase has coupled thereto polyriboadenylic acid (prA) and/or polydeoxyadenylic acid (pdA) template(s) by a ~~1-methylimidazole~~ coupling agent consisting essentially of 1-methylimidazole.

14(previously presented). The solid phase support of claim 13, wherein the solid phase is a microtiter plate having wells and an aliquot of a coupling solution, which comprises 100 mM 1-methylimidazole, pH \approx 5-7, and 0.5 - 2 mg/ml prA and/or pdA, is added to each well, followed by the incubation at a temperature of 10 - 60°C for 0.5 - 10 h, and washing each well for the removal of the 1-methylimidazole with the wash buffer, which comprises Bis-Tris propane, pH \approx 5-7, and drying and packaging the plates.

15(currently amended). The solid phase support of claim 14, wherein 100 μ l of the coupling solution, which ~~comprises~~ consist essentially of 100 mM 1-methylimidazole, pH \approx 6.25, and 1 mg/ml prA and/or pdA, is added to each well, followed by the incubation at room temperature for \approx 2 h, washing of each well with 2x300 μ l of the wash buffer, which comprises 10 mM Bis-Tris propane, pH \approx 6.25, drying the plates at 37°C for \approx 25 minutes and putting the plates in foil bags and vacuum sealing the bags.

16(withdrawn). A method of making a solid phase support for a Reverse Transcriptase (RT) assay kit containing solid phase bound polyriboadenylic acid (prA) and/or polydeoxyadenylic acid (pdA) template(s) which comprises contacting a polystyrene-based solid phase with a coupling solution comprising 1-methylimidazole, and prA and/or pdA, followed by incubation, washing with a wash buffer and drying.

17(withdrawn). The method of claim 16, wherein the solid phase is a microtiter plate and an aliquot of the coupling solution, which comprises 100 mM 1-methylimidazole, pH \approx 5-7, and 0.5 - 2 mg/ml prA and/or pdA, is added to each well, followed by the incubation at a temperature of 10 - 60°C for 0.5 - 10 h, and washing each well for the removal of the 1-methylimidazole with the wash buffer, which comprises Bis-Tris propane, pH \approx 5-7, and drying and packaging the plates.

18(withdrawn). The method of claim 17, wherein 100 μ l of the coupling solution, which comprises 100 mM 1-methylimidazole, pH \approx 6.25, and 1 mg/ml prA and/or pdA, is added to each well, followed by the incubation at room temperature for \approx 2 h, washing of each well with 2x300 μ l of the wash buffer, which comprises 10 mM Bis-Tris propane, pH \approx 6.25, drying the plates at 37°C for \approx 25 minutes and putting the plates in foil bags and vacuum sealing the bags.